THE EFFECTS OF COCAINE AND MORPHINE ALONE AND IN COMBINATION ON THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION

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Master of Arts

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THE EFFECTS OF COCAINE AND MORPHINE ALONE AND IN COMBINATION ON THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION

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Director of Thesis: 

The purpose of the current study was to determine whether suprathreshold doses of cocaine and morphine would combine in an additive fashion in the induction of behavioral sensitization. Therefore, rats were given one of four daily injections for 7 days: vehicle, cocaine (10 mg/kg), morphine (2.5 mg/kg), or a cocaine/morphine combination (10 mg/kg, 2.5 mg/kg) and tested for locomotor activity. In addition, on the 8th day, all groups were given a challenge injection of cocaine (10 mg/kg) and tested for activity. The results indicated that animals that received cocaine showed a progressive increase in locomotor activity (i.e., mean distance traveled) over the seven days and a greater response to the cocaine challenge than the vehicle treated animals. In contrast, the group that received morphine did not show sensitization over the 7 day pretreatment phase. However, on the cocaine challenge day, this group did indeed show a greater response to cocaine as compared to the control group. More importantly, during the pretreatment phase, the addition of cocaine to morphine did not increase activity as compared to controls over the pretreatment phase. Additionally, on day 8, the combination of the two drugs did not significantly

increase activity beyond either drug alone. Thus, the findings suggest that the combination of cocaine and morphine do not act in an additive manner.

Accepted by: _____ Chair

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Accepted by the faculty of the College of Education and Behavioral Sciences, Morehead State University, in partial fulfillment of the requirements for the Master of Arts degree.

Director of Thesis

Master's Committee: _____, Chair

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April 19, 2001
Date

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Special thanks to my family for their support and to Dr. Bruce A. Mattingly for his seemingly limitless patience.

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CHAPTER 1

INTRODUCTION

I. Introduction to behavioral sensitization

The abuse of psychostimulants (e.g., cocaine, amphetamines, morphine, etc.) has become an increasing problem over the last two decades (Robinson & Becker, 1986; Stewart & Badiani, 1993; Schifano, 1996). In humans, the acute administration of psychostimulant drugs produces an increase in arousal and states of euphoria. With repeated use, however, psychostimulants may produce an intense craving and a heightened sensitivity to other addictive drugs and drug-related stimuli. This increased sensitivity and craving often leads to compulsive drug seeking and drug taking behaviors (Schenk & Partridge, 1997; Robinson & Berridge, 2000; Robinson & Berridge, 2001). In addition, chronic psychostimulant abuse may also produce a variety of negative side effects such as anxiety, panic attacks, and schizophrenic-like psychoses (Robinson & Becker, 1986; Kalivas & Stewart, 1991; Robinson & Berridge, 1993). Although these negative side effects may subside if drug use is terminated, they may resurface up to ten years later if drug use is reinstated (Kalivas & Stewart, 1991). Therefore, it appears that relatively permanent neurological changes occur after chronic psychostimulant abuse.

In animals, intermittent injections of both direct (apomorphine) and indirect (e.g., cocaine, morphine, amphetamine) dopamine receptor agonists often produce a phenomenon known as behavioral sensitization that is characterized by a progressive

augmentation in a behavioral reaction (e.g., locomotion, self-administration, etc.) to a drug with repeated administration. An important feature of sensitization is that it appears to be a relatively permanent effect (Mattingly, Gotsick, & Marin, 1988). For example, animals pretreated with psychostimulants can exhibit a heightened sensitivity to subsequent injections of psychostimulants after days, weeks, or even months of drug withdrawal (Henry & White, 1991; Kalivas & Duffy, 1987; Mattingly et al., 1988; Vanderschuren et al., 1999; Vanderschuren et al., 2000). Recent evidence suggests that behavioral sensitization develops to both the locomotor activating and reinforcing effects of most drugs (Mattingly et al., 1988; Kalivas & Duffy, 1987; Schenk & Partridge, 1997; Robinson & Becker, 1986; Robinson & Berridge, 1993; Stewart & Badiani, 1993). Thus, the sensitization observed in animals and the side effects of long-term psychostimulant abuse observed in humans may be related to a common neurobiological mechanism (see Robinson & Becker, 1986; Kalivas & Stewart, 1991; Robinson & Berridge, 1993, for review). Consequently, a great deal of research has been directed at determining the neurobiological mechanisms mediating the development of behavioral sensitization.

II. Relationship of the dopaminergic system to cocaine and /or opiate-induced behavioral sensitization

Although behavioral stimulants affect a variety of neurochemical systems in the brain, the locomotor activating and reinforcing effects of behavioral stimulants are thought to be mediated by the dopaminergic system (see Robinson & Becker, 1986;

Kalivas & Stewart, 1991; Robinson & Berridge, 1993; Robinson & Berridge, 2000; Robinson & Berridge, 2001).

The mesocorticolimbic system (originating from the ventral tegmental area and projecting to the limbic system and pre-frontal cortex) is commonly associated with both the rewarding and locomotor activation aspects of drugs of abuse while the nigrostriatal system is associated with other motoric effects of these drugs (White, 1996; Robinson & Berridge, 1993).

Both the mesocorticolimbic and nigrostriatal system appear to play a definitive role in both the development and persistence of behavioral sensitization to cocaine and morphine. Indeed, repeated cocaine or morphine treatments cause similar changes in both systems suggesting a common underlying neural mechanism responsible for the development of behavioral sensitization to these psychostimulants (Beitner-Johnson, Guitart, & Nestler, 1992).

For example, chronic exposure to cocaine or morphine produces long-term adaptations in G-proteins, adenylyl cyclase activity, cAMP-dependent protein kinase, and tyrosine hydroxylase levels in the ventral tegmental area (VTA) and/or the nucleus accumbens (Beitner-Johnson, Guitart, & Nestler, 1992). Both chronic morphine and cocaine treatments increase the tyrosine hydroxylase level in the ventral tegmental area but have no effect in the nucleus accumbens, substantial nigra, or the caudate putamen. The increase in tyrosine hydroxylase activity may reflect a common change in the ventral tegmental area and a general increase in VTA neuronal

activity (Beitner-Johnson et al., 1992). Indeed, neurochemical lesions of the VTA and/or the nucleus accumbens have been shown to disrupt both opiate and cocaine self-administration. Also, animals will self-administer these drugs into these brain regions suggesting a common mechanism within the mesocorticolimbic system mediating the rewarding aspect of cocaine and morphine (Beitner-Johnson et al., 1992). In addition, Kalivas and Duffy (1987) demonstrated that repeated injections of opioids into the A10 dopamine region produce a dopamine dependent increase in motor activity. Daily intra-A10 administration of opioids is associated with an augmented increase in dopamine metabolites in the nucleus accumbens. Therefore, changes in dopaminergic neurotransmission appear to be involved in mediating some of the behavioral effects of opioids.

In addition to the mesocorticolimbic system, parts of the nigrostriatal system appear to play a significant role in the development of behavioral sensitization to cocaine or morphine. Chronic morphine has been shown to increase DOPAC levels, homovillic acid levels, and the dopamine turnover rate in the striatum (Airio & Ahtee, 1997). Kalivas and Stewart (1991) demonstrated that behavioral sensitization to repeated administration of opioids related to the indirect stimulation of dopamine cell bodies in the VTA and the substantia nigra.

Therefore, several common mechanisms appear to exist for the development of behavioral sensitization to either opiates or cocaine.

III. Relationship of dopamine receptors to cocaine and/or opiate-induced behavioral sensitization

Although one line of research investigates the anatomical regions in relation to behavioral sensitization, another line of research studies the involvement of dopaminergic receptors in the development of behavioral sensitization.

Recently, it has been discovered that dopamine (DA) receptors have five different subtypes, which are classified under the D1 and D2 subfamilies depending upon the receptor's biochemical, molecular, and pharmacological properties (Sibley, Monsma, & Shen, 1993; O'Dowd, 1993). The D1 subfamily includes the D1 and D5 receptor subtypes which, when activated, stimulate adenylate cyclase activity. The D2 subfamily includes the D2, D3, and D4 receptor subtypes. The dopamine D2 subfamily receptors do not promote or inhibit adenylate cyclase activity.

Research has shown that most stimulant type drugs either directly or indirectly produce an overstimulation of DA receptors in both the D1 and D2 receptor sub-families (Ferger, Kropf, & Kuschinsky, 1994). In addition, a number of changes in dopamine receptor function have been reported following the chronic administration of psychostimulants in rats (Kalivas & Stewart, 1991).

A supersensitivity has been reported in D1 post-synaptic receptors following chronic cocaine administration (Henry, Green, & White, 1989; Henry & White, 1991). Importantly, the D1 supersensitivity is still apparent following several weeks of drug withdrawal (Henry & White, 1991). In addition, the co-administration of a

D1 antagonist with cocaine decreases cocaine self-administration and prevents sensitization to the conditioned rewarding effects of cocaine (Caine & Koob, 1994, Shippenberg & Heidbreder, 1995).

Unlike the supersensitivity found in the D1 receptors, repeated cocaine treatments induce sub-sensitivity in dopamine D2 autoreceptors (Henry et al., 1989; Henry and White, 1991). However, this decrease in autoreceptor sensitivity is transient, lasting only a few days after drug withdrawal (Ackerman and White, 1990). It is also important to note that the co-administration of a D2 antagonist with cocaine has no effect on the self-administration or conditioned place preference to cocaine (Caine & Koob, 1994; Shippenberg & Heidbreder, 1995). Thus, it would appear that the D1 receptor is the controlling factor in the development of behavioral sensitization to the rewarding effects to cocaine. However, recent evidence has demonstrated that neither the D1 receptor nor the D2 receptor alone controls the development of behavioral sensitization to the locomotor stimulating effect of cocaine (Mattingly et al., 1994; White et al., 1998).

In addition to cocaine, behavioral sensitization has been shown to develop from the repeated administration of the mu opioid receptor agonist, morphine. Morphine-induced behavioral sensitization may be due to indirect stimulation of dopamine receptors leading to an increase in extracellular dopamine (Di Chiara & Imperato, 1988). Like cocaine sensitization, studies indicate that both D1 and D2 receptor stimulation is needed for the expression but not for the development of

behavioral sensitization to systemically administered morphine (Jeziorski & White, 1995). Also, it has been shown that pretreatment with dopamine agonists can either enhance or reduce morphine-induced locomotion. For example, in mice, the intra-cerebral co-administration of the D1-agonist, SKF38393, increased morphine-induced locomotion by approximately 2-fold which was antagonized by the D1 antagonist, SCH23390. On the contrary, the intra-cerebral co-administration of a low dose of the D2 agonist quinpirole, which may stimulate D2 autoreceptor activity, and morphine resulted in a reduction of morphine-induced locomotion (Funada, Suzuki, & Misawa, 1994). In addition, withdrawal from repeated morphine increases the sensitivity of dopamine D2-like receptors (Piepponen et al., 1996). Therefore, dopamine receptors may play a role in the expression, acceleration, and overall increase in sensitivity to morphine (Jeziorski & White, 1995; Kuribura, 1995).

IV. Cross-sensitization between cocaine and morphine

Although most research has focused on the development of behavioral sensitization to the repeated administration of a single psychostimulant, several studies have either directly or indirectly investigated cross-sensitization. Cross-sensitization is similar to behavioral sensitization in that there is an augmented response to a previous drug exposure. However, the difference between cross-sensitization and behavioral sensitization is the animal shows an enhanced response to a drug due to previous exposure to different drug. For example, opioid and dopamine

agonists have been shown to enhance each other's locomotor activating and reward effects (Vanderschuren et al., 1997).

In addition to locomotion, cross-sensitization to the rewarding effects of opioids and dopamine agonists has been demonstrated. For example, dopamine agonists (i.e., amphetamine and cocaine) and morphine were shown to cross-sensitize to each other in tasks measuring conditioned reward (Shippenberg & Heidbreder, 1995; Venzina et al., 1989; Shippenberg, LeFevour, & Thompson, 1998; Cunningham & Kelley, 1992).

In addition to cross-sensitivity between drugs, recent studies suggest that the combined administration of drugs of different classes, particularly dopamine agonists and opioid agonists, may increase the behavioral and rewarding aspects of these drugs in an additive or supra-additive way. Recently, the combination of cocaine with morphine or heroin, commonly known as "speedball", has received a great amount of attention. Several studies have investigated "speedball" and have produced conflicting results. For example, Mello et al. (1995) found that the effects of cocaine and heroin combinations on drug self-administration did not differ from that of either drug alone. In contrast, several other studies have demonstrated that the "speedball" combination demonstrates an enhancement in the rewarding effects as compared to the rewarding effects of either drug alone (Rowlett & Woolverton, 1997; Duvauchelle, Sapoznik, & Kornetsky, 1998). The conflicting results between studies may reflect differences in experimental design such as varying schedules of

reinforcement.

In addition to the reinforcing aspects of "speedball", the locomotor effects of the cocaine and opioid agonist combinations have been investigated. Indeed, several studies have demonstrated that the "speedball' combination typically produces an enhanced acute locomotor response compared to that of either drug alone (Kunko, French, & Izenwasser, 1998; Kimmel & Holtzman, 1997; Kimmel, Tallarida, & Holtzman, 1997).

Although opioid/cocaine combinations appear to enhance the acute locomotor activating effects of either drug, repeated treatments with morphine and cocaine do not appear to enhance the development of behavioral sensitization (Mattingly et al., 1999). However, this latter study used doses of cocaine and morphine individually subthreshold for the development of behavioral sensitization. Consequently, whether high suprathreshold doses of cocaine and morphine would combine in an additive fashion in the development of behavioral sensitization is unknown.

V. Purpose

In summary, repeated treatments with either cocaine or morphine produce behavioral sensitization. In addition, cross-sensitization between these two drugs has been reported. Further, the rewarding and acute locomotor effects appear to combine in an additive or supra-additive fashion. At present, the nature of the interaction between cocaine and opiates on the development of behavioral sensitization is

unclear. The purpose of the present study, therefore, was to determine whether suprathreshold doses of cocaine and morphine would combine in an additive fashion in the induction of behavioral sensitization.

Consequently, groups of rats were given injections of vehicle, cocaine, morphine, or a cocaine/morphine combination every forty-eight hrs and then tested for locomotor activity. At the conclusion of this chronic pretreatment, all animals were given a challenge injection of cocaine to test for behavioral sensitization and cross-sensitization.

CHAPTER 2

METHODS

Subjects

Thirty-two adult male Wistar albino rats (Harlan Industries, Indianapolis, IN) weighing 250-300 grams served as subjects. All rats were individually housed in hanging wire-mesh cages in a temperature- controlled colony room with a 12 h light/dark cycle. Animals were given unrestricted access to food and water. All behavioral testing was conducted during the light phase of the cycle.

Apparatus

Activity measures were taken in four square (approximately 41 X 41 cm) open field test chambers (MED Associates, model OFA-163). The base of each chamber was 1.3 cm thick, white PVC. The sidewalls were 0.6cm clear acrylic with the corners reinforced by aluminum angle. The chambers were equipped with a 16X16 array of infrared photocell beams located 2.5 cm above the floor and a single array of 16 photocells 10 cm above the floor. In addition, a clear acrylic cylinder (41 cm diameter) was placed inside each test chamber (see Figure 1).

Output from each photocell array was connected to a Gateway 2000 computer in an adjacent room through a MED Associates interface. Using MED Associates software, distance traveled (cm), stereotypic count (small movements), and vertical count (rearing), were measured and stored during the test session.

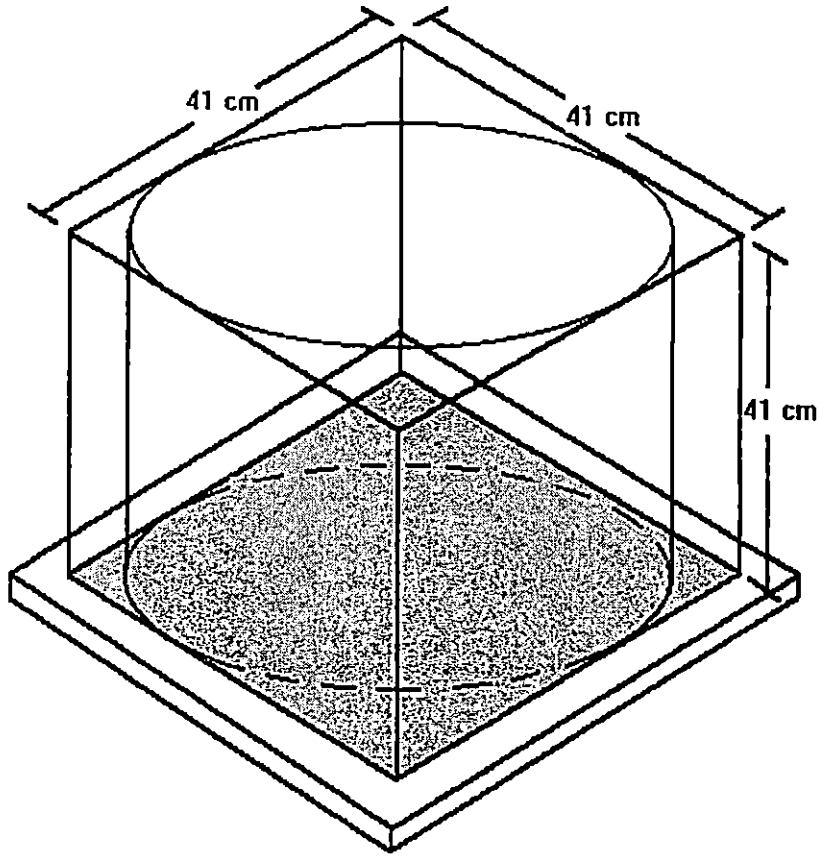

Figure 1. Med-Associates locomotor activity chamber

Drugs

Cocaine hydrochloride (N.I.D.A.) and/or Morphine sulfate (N.I.D.A.) were dissolved daily into distilled H_2O and injected IP in a volume of 1.0 ml/kg. All doses were calculated based on salt weight of the drug. Vehicle injections were given using the same route and volume as the corresponding drug injection.

Design and Procedure

At the beginning of the experiment, the rats were randomly assigned to one of four groups (8 animals per group) comprising a two (vehicle vs. cocaine) X two (vehicle vs. morphine) factorial design. Therefore, the rats were divided into four pretreatment groups: vehicle/vehicle, vehicle/cocaine, morphine/vehicle, and morphine/cocaine (see Table 1). The experimental groups were counterbalanced between the four activity chambers (See Appendix A, Table 11).

Table 1. Experimental Design

Experimental groups (2 X 2 factorial design)

	VEHICLE	MORPHINE
VEHICLE	Vehicle (n = 8)	Morphine (n = 8)
COCAINE	Cocaine (n = 8)	Morphine/Cocaine (n = 8)

The experiment was conducted in two phases: a pre-exposure phase and a cocaine sensitization test. During the pre-exposure phase of the experiment, rats were

injected with a single injection of vehicle, cocaine (10 mg/kg), morphine (2.5 mg/kg), or a cocaine/morphine (10 mg/kg, 2.5 mg/kg) cocktail. After the daily injection, each rat was given five min for the drug to take effect and then tested for locomotor activity for 120 min. This injection-test procedure was repeated every 48 hrs for a total of seven sessions. Twenty-four hrs after the last pre-exposure session, the cocaine sensitization test phase was initiated. During this test, all rats were given a 10 mg/kg injection of cocaine, a 5 min waiting period, and then a 120 min sensitization test for locomotor activity.

Data Analysis

Each measure of motor activity was recorded in blocks of 10 min and analyzed using a mixed-factor analysis of variance with drug treatment conditions as between groups factors and activity test sessions and blocks of 10 min within sessions as repeated measures. Significant interactions were analyzed with additional ANOVAs performed on individual sessions and/or block data, followed by Neuman-Keuls post hoc tests.

CHAPTER 3

RESULTS

Pretreatment Sessions (Days 1-7)

Distance Traveled:

The mean distance traveled in centimeters for each of the four pretreatment groups for the seven 120 min pretreatment sessions is illustrated in Figure 2. The within session activity across twelve 10 min blocks for the four pretreatment groups on Day 1, 4, and 7 is depicted in Figure 3. A mixed factor analysis of variance was performed on the mean distance traveled data with drug treatment conditions as between-groups factors and activity test sessions and blocks within sessions as repeated measures (See Appendix A, Table 2).

As may be seen in Figure 2, overall rats treated with cocaine (i.e., Vehicle-Cocaine group, Morphine-Cocaine group) were significantly more active than the vehicle treated rats (i.e., Vehicle-Vehicle group) across the pretreatment sessions, [cocaine effect: $F(1, 28) = 33.41$, $p < .0001$]. Moreover, as seen in Figures 2 and 3, this cocaine effect increased across days and was greatest during the first few blocks of each session [Cocaine x Day interaction: $F(6, 168) = 3.63$, $p < .01$; Cocaine x Block interaction: $F(11, 308) = 48.15$, $p < .0001$; Cocaine x Day x Block interaction: $F(66, 1848) = 2.72$, $p < .0001$]. Similar to cocaine, rats pretreated with morphine tended to be more active than vehicle rats [morphine effect: $F(1, 28) = 9.71$, $p <$

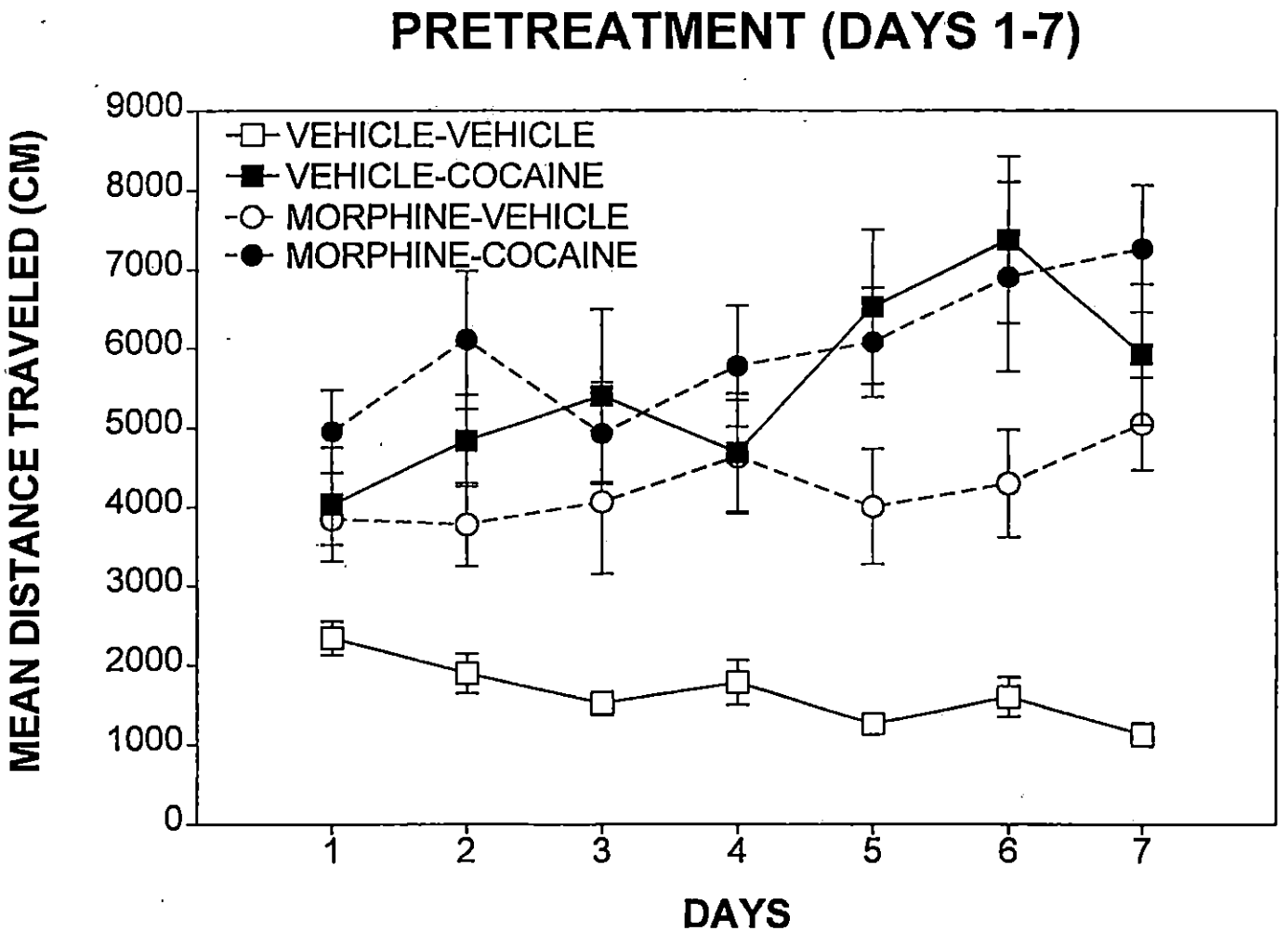

Figure 2. Mean distance traveled in cm (± SEM) for each of the four pretreatment groups over the seven 120 min pretreatment sessions.

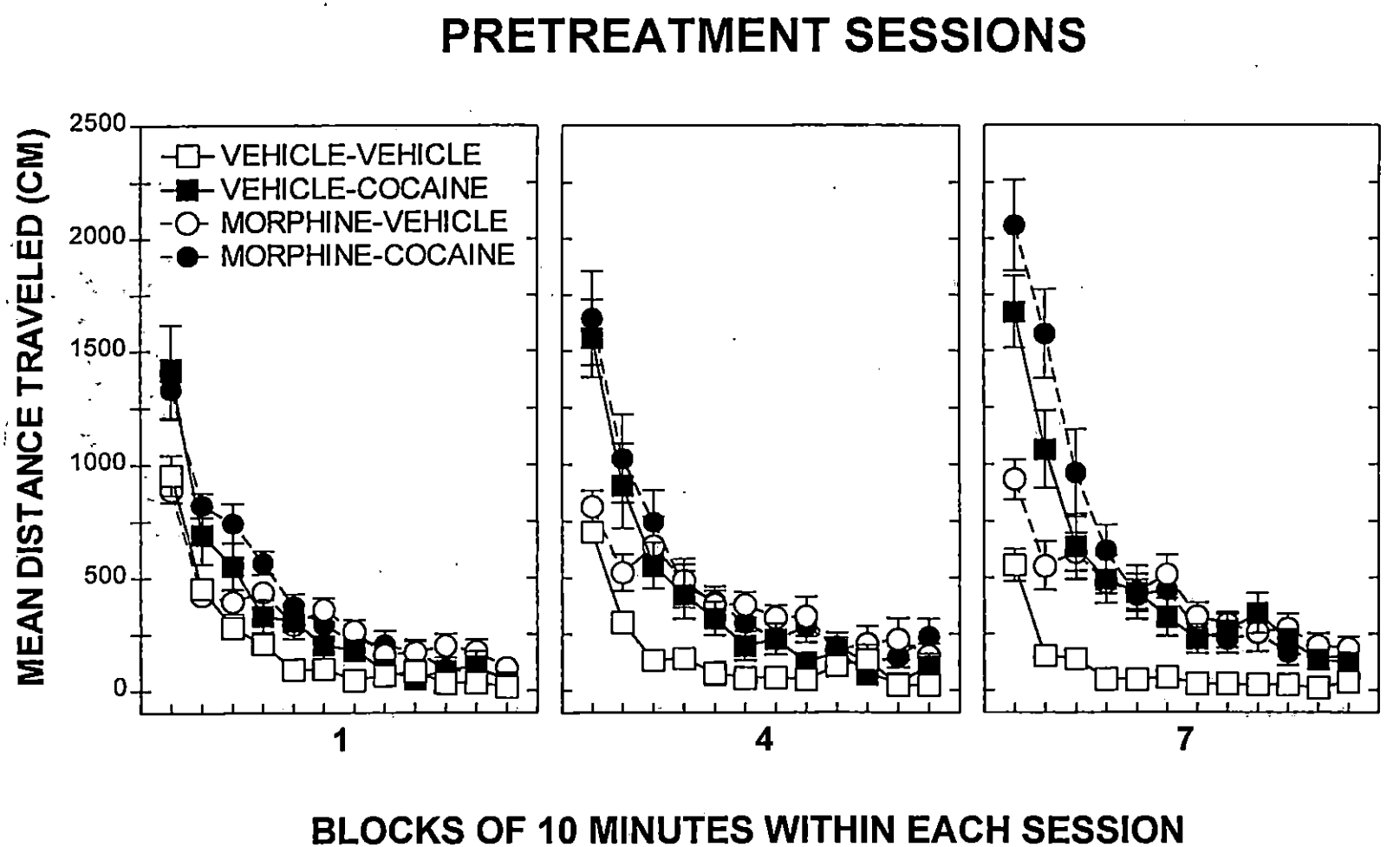

Figure 3. Mean distance traveled in cm (± SEM) for each of the four pretreatment groups across the twelve 10 min blocks within each of the 120 min pretreatment sessions for days 1, 4, and 7.

.001]. However, morphine had a significantly greater overall effect on rats given vehicle than for rats pretreated with cocaine [Morphine x Cocaine interaction: $\underline{F}(1, 28) = 4.74$, $\underline{p} < .05$]. Indeed, for rats pretreated with vehicle, morphine significantly increased activity [Newman-Kuels, $\underline{p} < .05$]. However, morphine did not significantly increase activity for rats treated with cocaine suggesting that an additive effect does not exist between cocaine and morphine (i.e., Morphine-Cocaine group vs Vehicle-Cocaine group; Newman-Kuels, $\underline{p} > .05$].

Stereotypic Counts:

The mean stereotypic counts for each of the four pretreatment groups for the seven 120 min pretreatment sessions is illustrated in Figure 4. The within session activity across twelve 10 min blocks for the four pretreatment groups on days 1, 4, and 7 is depicted in Figure 5. A mixed factor analysis of variance was performed on the stereotypic counts data with drug treatment conditions as between-groups factors and activity test sessions and blocks within sessions as repeated measures (See Appendix A, Table 3).

As illustrated in Figure 4, overall, rats pretreated with cocaine were significantly more active than animals that did not receive cocaine [cocaine effect: $\underline{F}(1,28) = 41.08$, $\underline{p} < .0001$] and this effect was more pronounced in the early blocks [Cocaine x Block interaction: $\underline{F}(11, 308) = 34.99$, $\underline{p} < .0001$]. Rats pretreated with

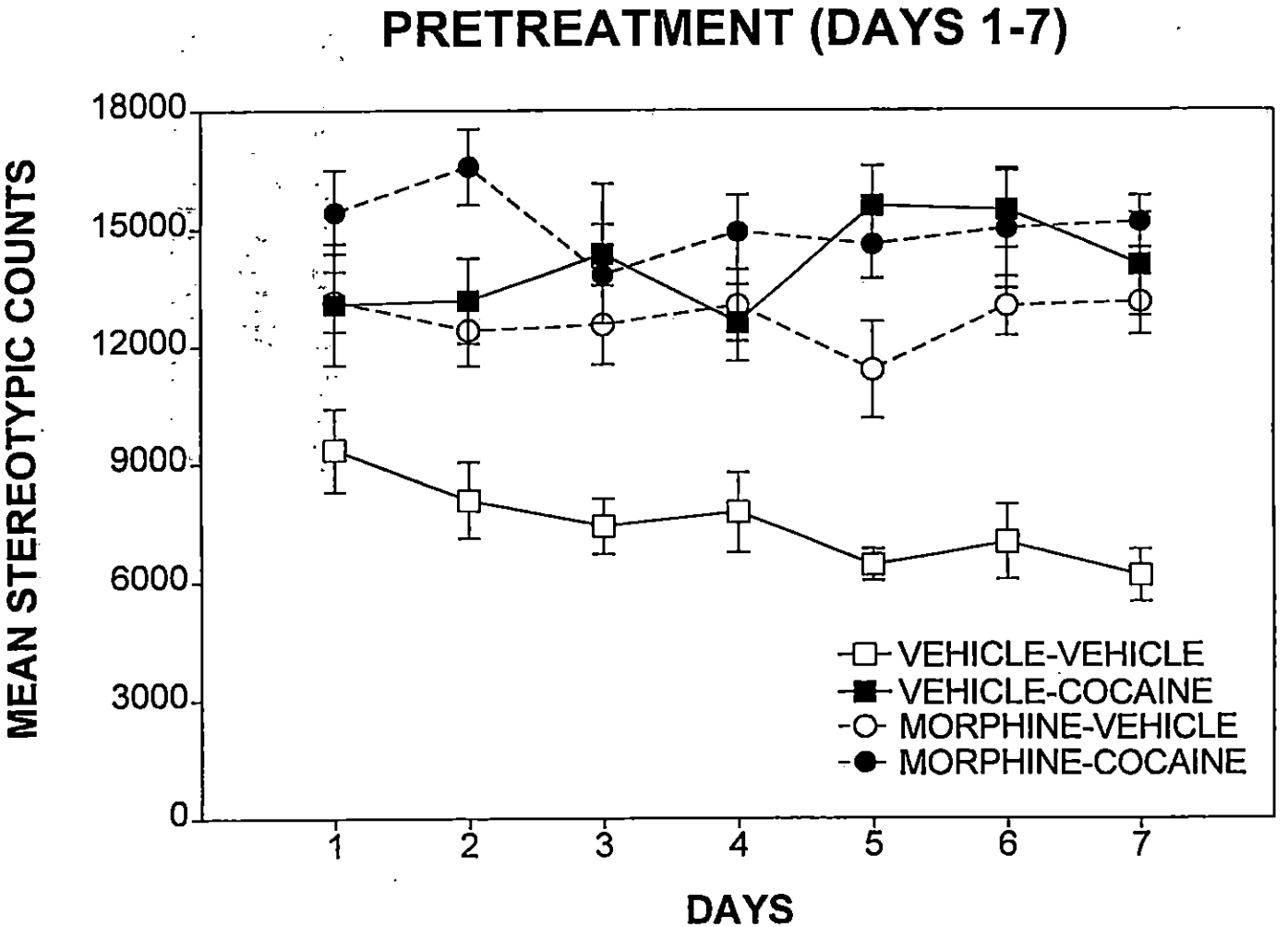

Figure 4. Mean stereotypic counts for each of the four pretreatment groups over the seven 120 min pretreatment sessions.

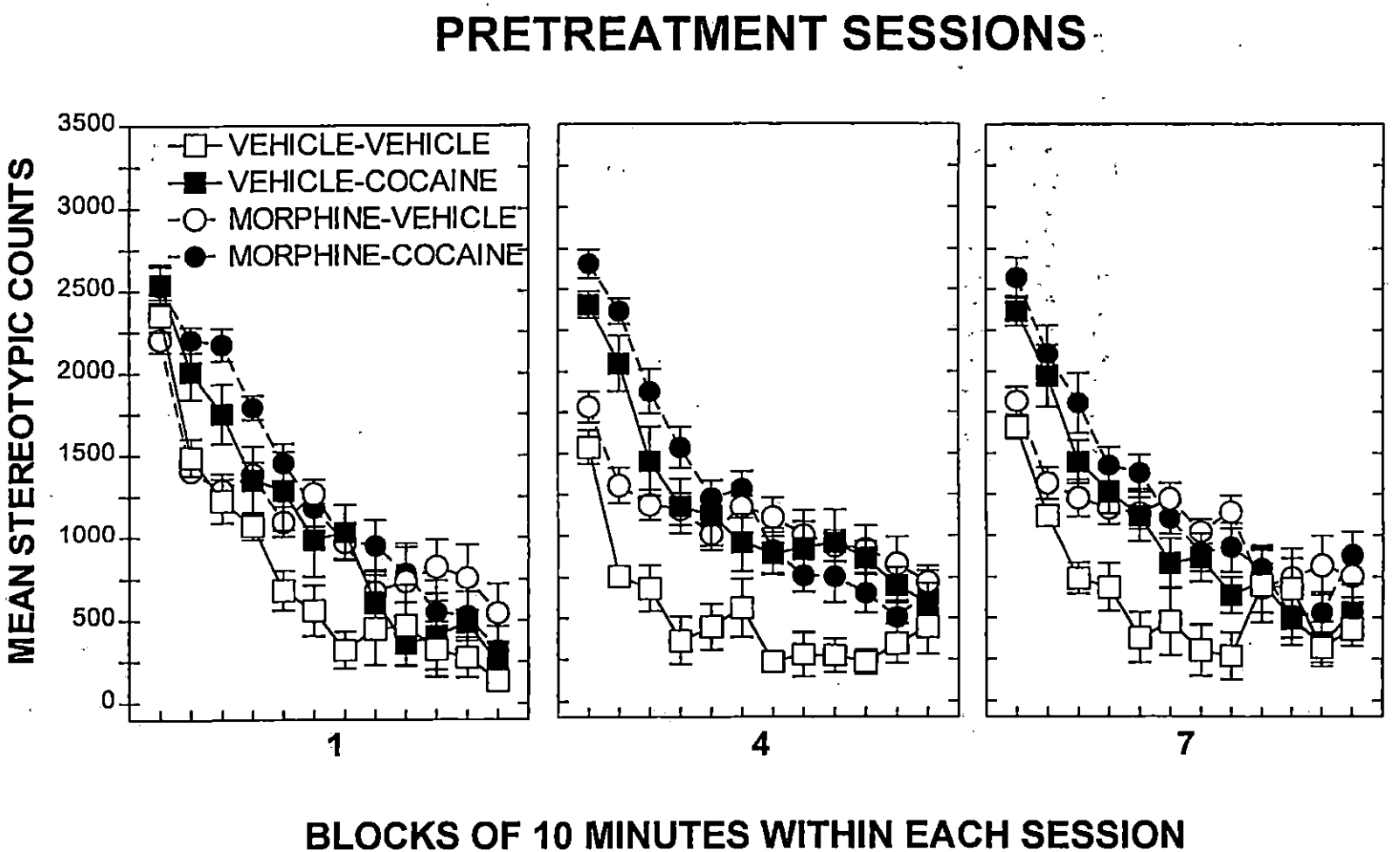

Figure 5. Mean stereotypic counts for each of the four pretreatment groups across the twelve 10 min blocks within each of the 120 min pretreatment sessions for days 1, 4, and 7.

morphine also displayed increased stereotypy counts compared to vehicle treated animals over the seven days, particularly on the middle blocks of each session [morphine effect: $\underline{F}(1, 28) = 19.72$, $\underline{p} < .0001$; Morphine X Block interaction: $\underline{F}(11, 308) = 2.20$, $\underline{p} < .01$]. However, cocaine did not increase stereotypy in morphine pretreated animals and morphine did not increases stereotypy in cocaine pretreated animals [Morphine x Cocaine interaction: $\underline{F}(1, 28) = 8.87$, $\underline{p} < .01$; Newman-Kuels test, $\underline{p} > .05$]. In addition, neither drug produced a progressive increase in stereotypy across sessions [Cocaine X Day interaction: $\underline{F}(6, 168) = 1.73$, $\underline{p} > .05$; Morphine X Day interaction: $\underline{F} < 1.00$; Morphine x Cocaine x Day interaction: $\underline{F}(6, 168) = 1.96$, $p > .05$].

Rearing:

The mean number of rears for each of the four pretreatment groups for the seven 120 min pretreatment sessions is illustrated in Figure 6. The within session activity across twelve 10 min blocks for the four pretreatment groups on Day 1, 4, and 7 is depicted in Figure 7. A mixed factor analysis of variance was performed on the rearing data with drug treatment conditions as between-groups factors and activity test sessions as repeated measures (See Appendix A, Table 4). As seen in Figure 6, overall, cocaine significantly increased rearing compared to animals not receiving cocaine [cocaine effect: $\underline{F}(1, 28) = 42.97$, $\underline{p} < .0001$]. In addition, as depicted in

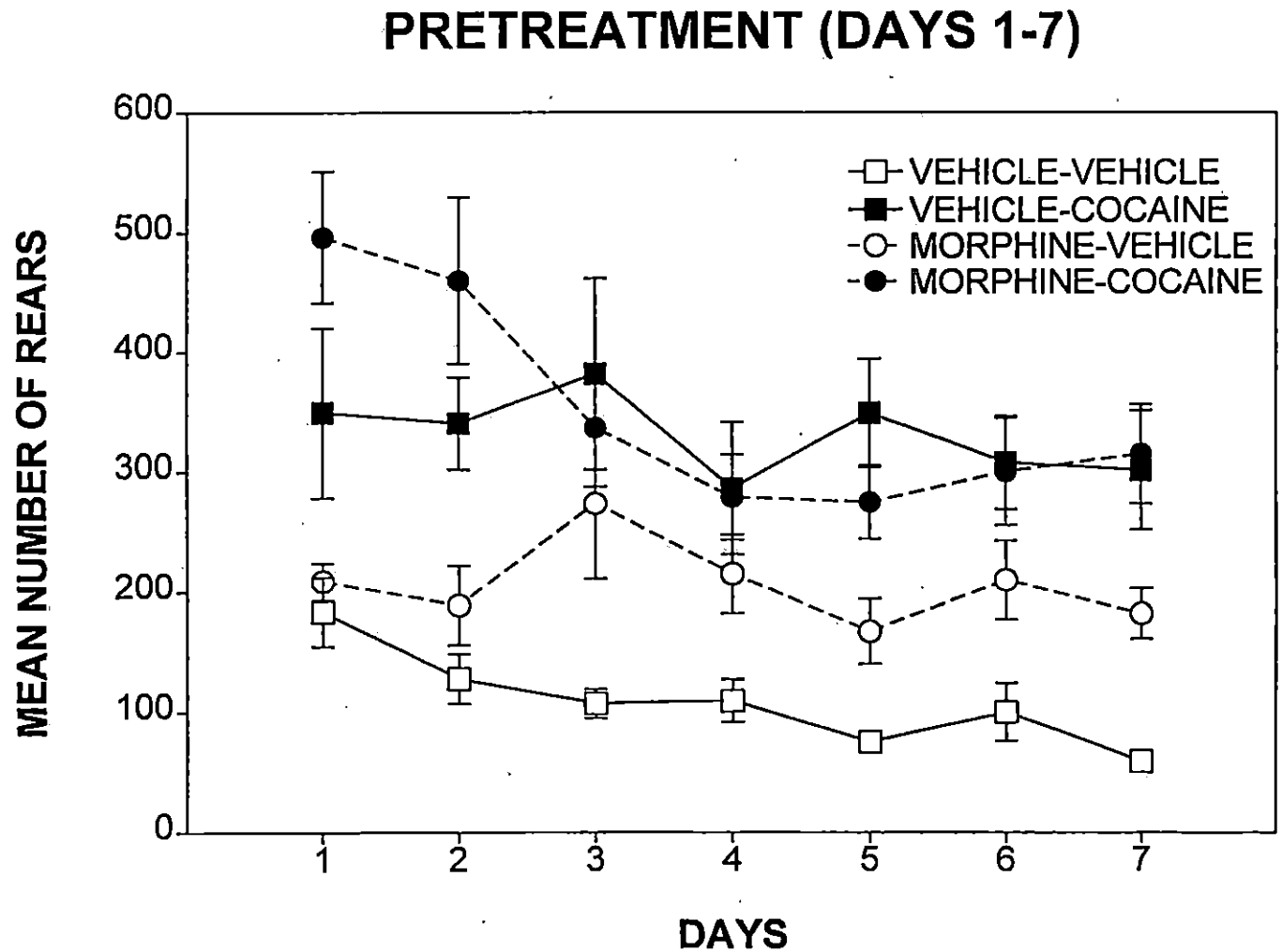

Figure 6. Mean number of rears for each of the four pretreatment groups over the seven 120 min pretreatment sessions.

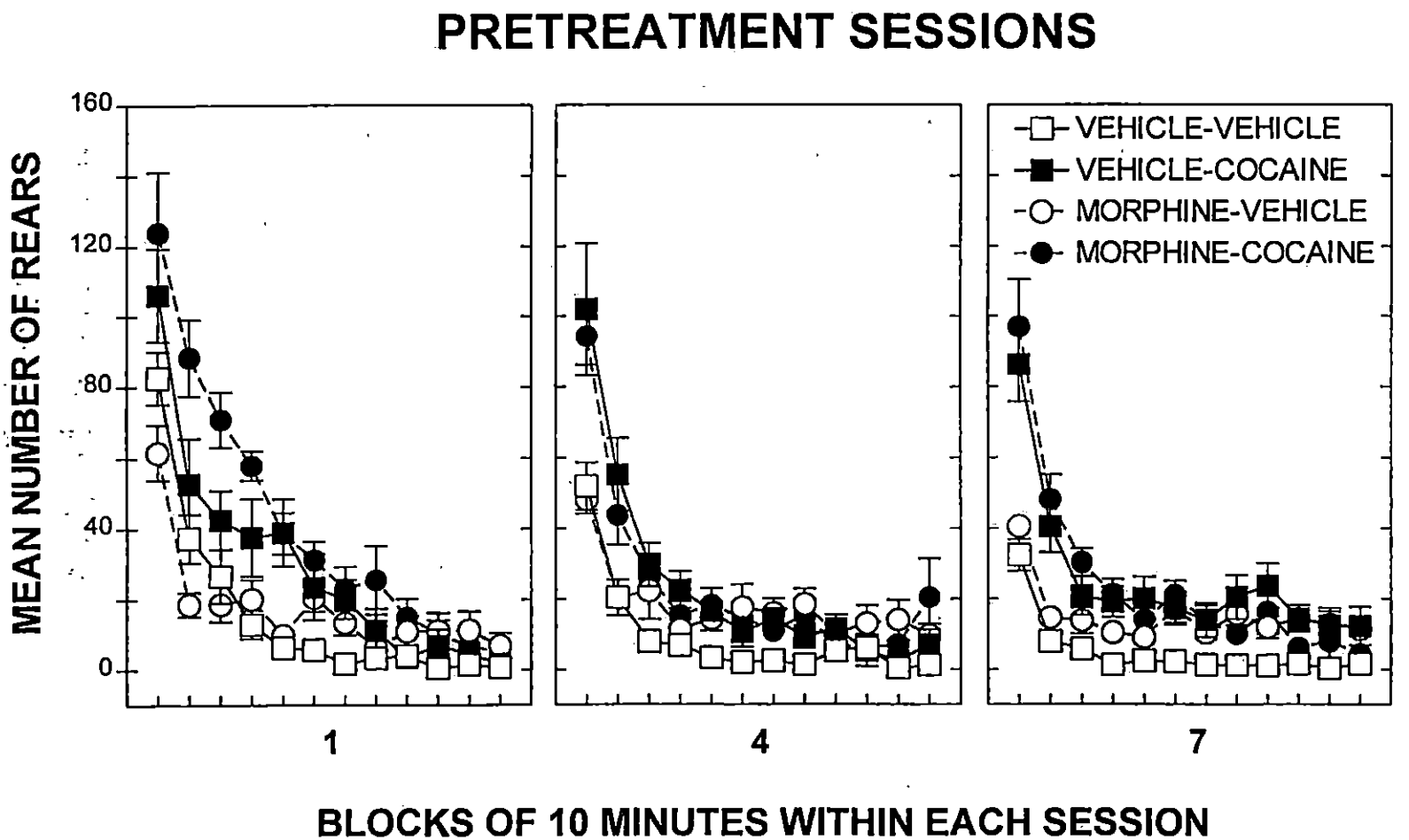

Figure 7. Mean number of rears for each of the four pretreatment groups across the twelve 10 min blocks within each of the 120 min pretreatment sessions for days 1, 4, and 7.

Figure 7, this cocaine-induced increase in rearing appeared greatest during the early blocks and dissipated during these blocks across the seven days [Cocaine x Block interaction: $F(11, 308) = 29.90$, $p < .0001$; Cocaine x Day x Block interaction; $F(66, 1848) = 1.60$, $p < .0001$].

Morphine also significantly increased overall rearing across the seven days compared to vehicle rats [morphine effect: $F(1, 28) = 4.28$, $p < .05$], but this increase did not vary across days or blocks [Morphine x Day interaction: $F(6, 168) = 0.63$, $p > .05$; Morphine x Block interaction: $F(11, 308) = 0.44$, $p > .05$; Morphine x Day x Block interaction: $F(66, 1848) = 0.92$, $p > .05$].

Cocaine Challenge (Day 8)

Distance Traveled:

A mixed factor analysis of variance was performed on the mean distance traveled data with drug treatment conditions as between-groups factors and blocks within sessions as repeated measures (See Appendix A, Table 5). The mean distance traveled for the four pretreatment groups following a 10 mg/kg challenge injection of cocaine is shown in Figure 8 and the within session mean distance traveled activity of the pretreatment groups is shown in Figure 9.

As may be seen in Figure 8, overall, rats pretreated with cocaine were significantly more active following a challenge injection of cocaine compared to rats with no previous exposure to cocaine [cocaine effect: $F(1, 28) = 10.50$, $p < .01$]. This

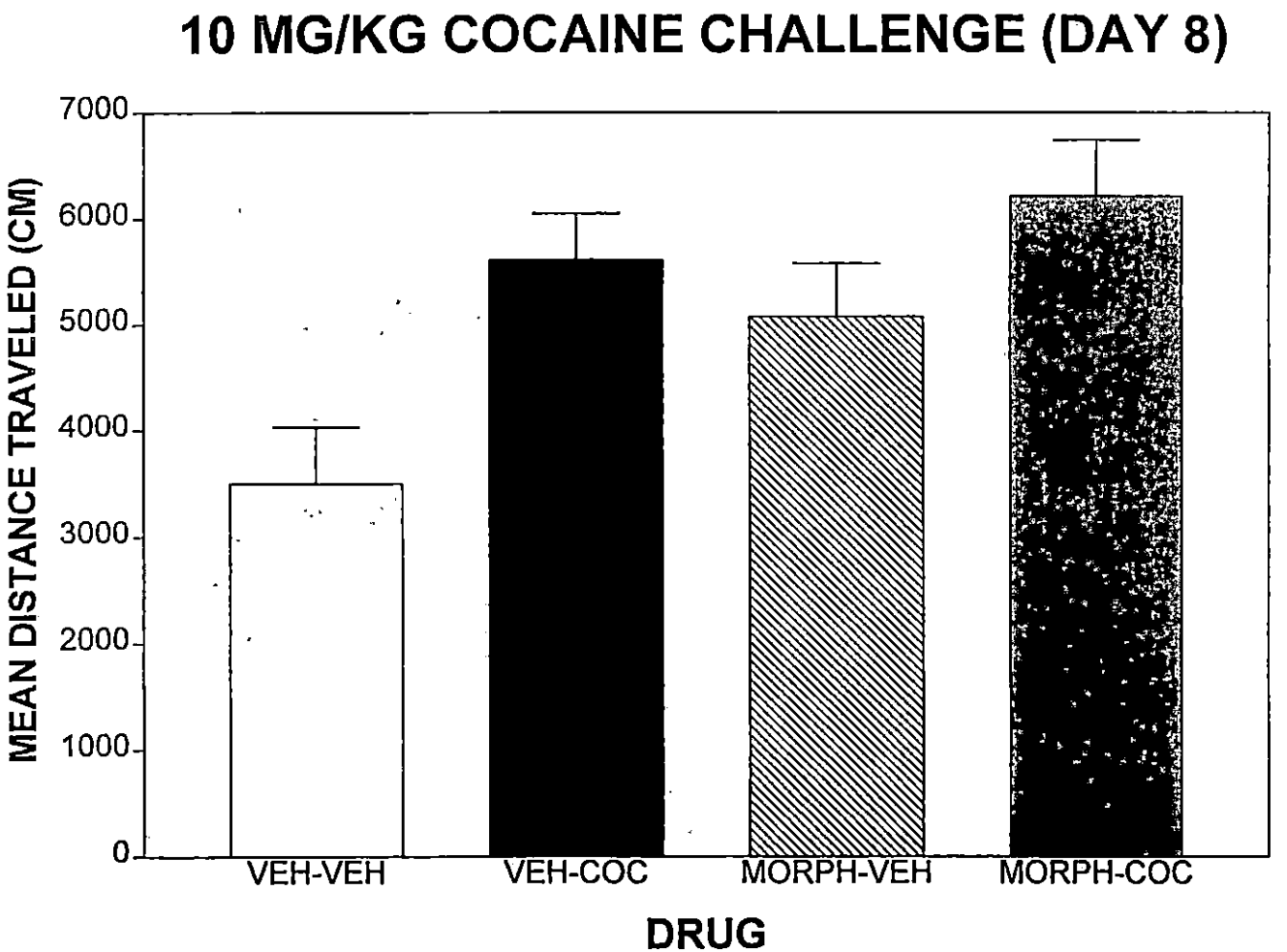

Figure 8. Mean distance traveled in cm (± SEM) after a challenge injection of cocaine (10 mg/kg) for each of the four pretreatment groups over the 120 min session.

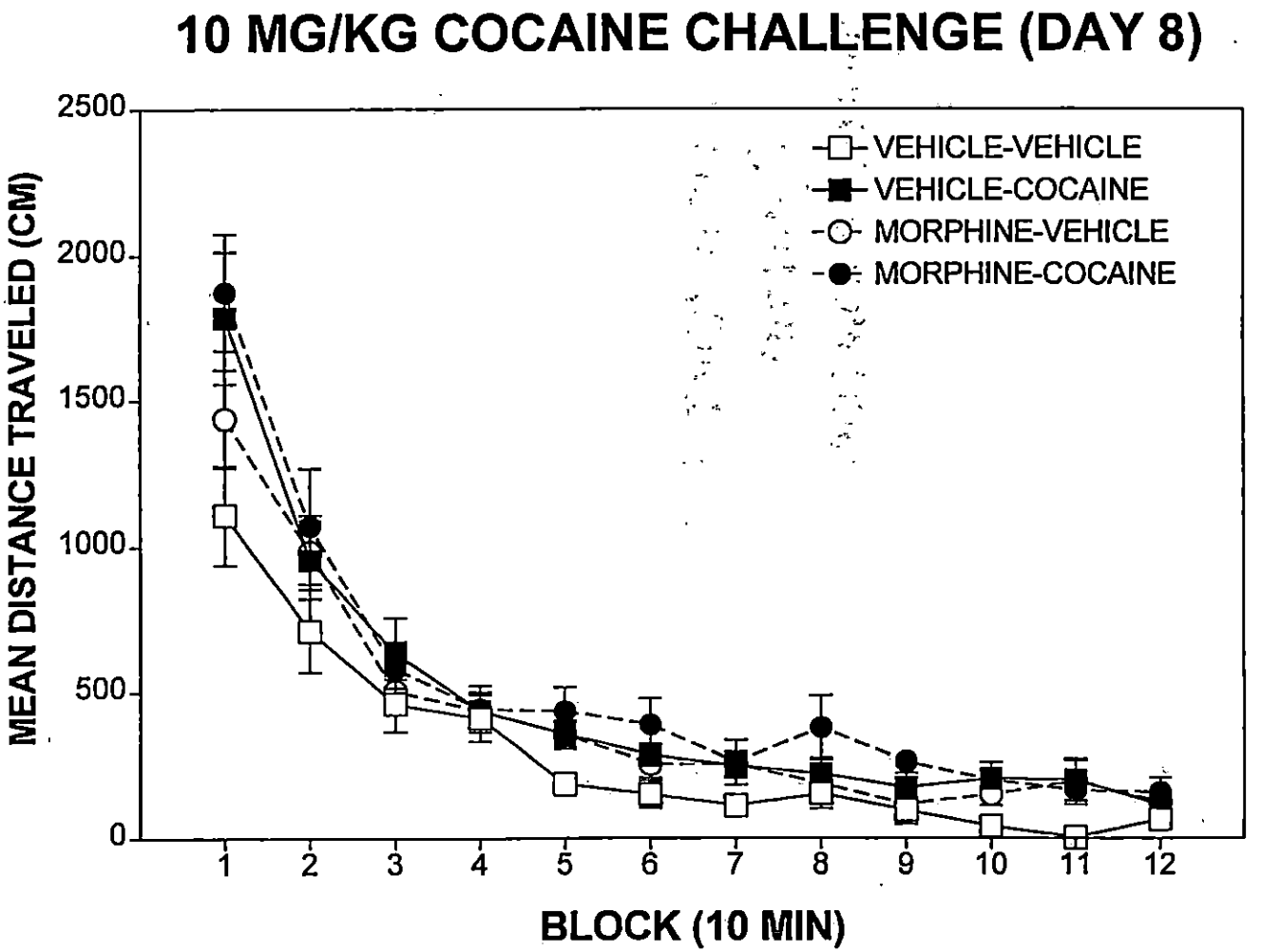

Figure 9. Mean distance traveled in cm (± SEM) after a challenge injection of cocaine (10 mg/kg) for each of the four pretreatment groups across the twelve 10 min blocks within the 120 min session.

cocaine-induced increase in activity decreased across blocks [cf. Figure 9; block effect: $\underline{F}(11, 308) = 105.48$, $\underline{p} < .0001$; Cocaine x Block interaction: $\underline{F}(11, 308) = 2.84$, $\underline{p} < .01$]. In addition to cocaine, overall, rats pretreated with morphine (i.e., Morphine-Vehicle group and the Morphine-Cocaine group) were significantly more active following a challenge injection of cocaine than rats pretreated with vehicle (i.e., Vehicle-Vehicle and the Vehicle-Cocaine) [morphine effect: $\underline{F}(1, 28) = 4.69$, $\underline{p} < .05$]. The Morphine x Cocaine interaction was not significant, $\underline{p} = 0.96$. Thus, both morphine and cocaine pretreatment produced a subsequent increase in sensitivity to cocaine. However, a planned comparison between the vehicle/cocaine and the morphine/cocaine pretreated groups revealed no significant differences in distance traveled following the cocaine challenge injection [$t(14) = 0.401$, $p>.05$]. Similarly, the morphine/cocaine pretreatment did not significantly differ in activity from the morphine/vehicle group on this test [$t(14) = 0.143$, $p>.05$]. Thus, although both cocaine and morphine pretreatment increased sensitivity to cocaine, the increase was not additive.

Stereotypic Counts:

The mean stereotypic counts for the four pretreatment groups during the 120 min cocaine challenge session are shown in Figure 10 and the within session stereotypic activity for the four pretreatment groups is illustrated in Figure 11.

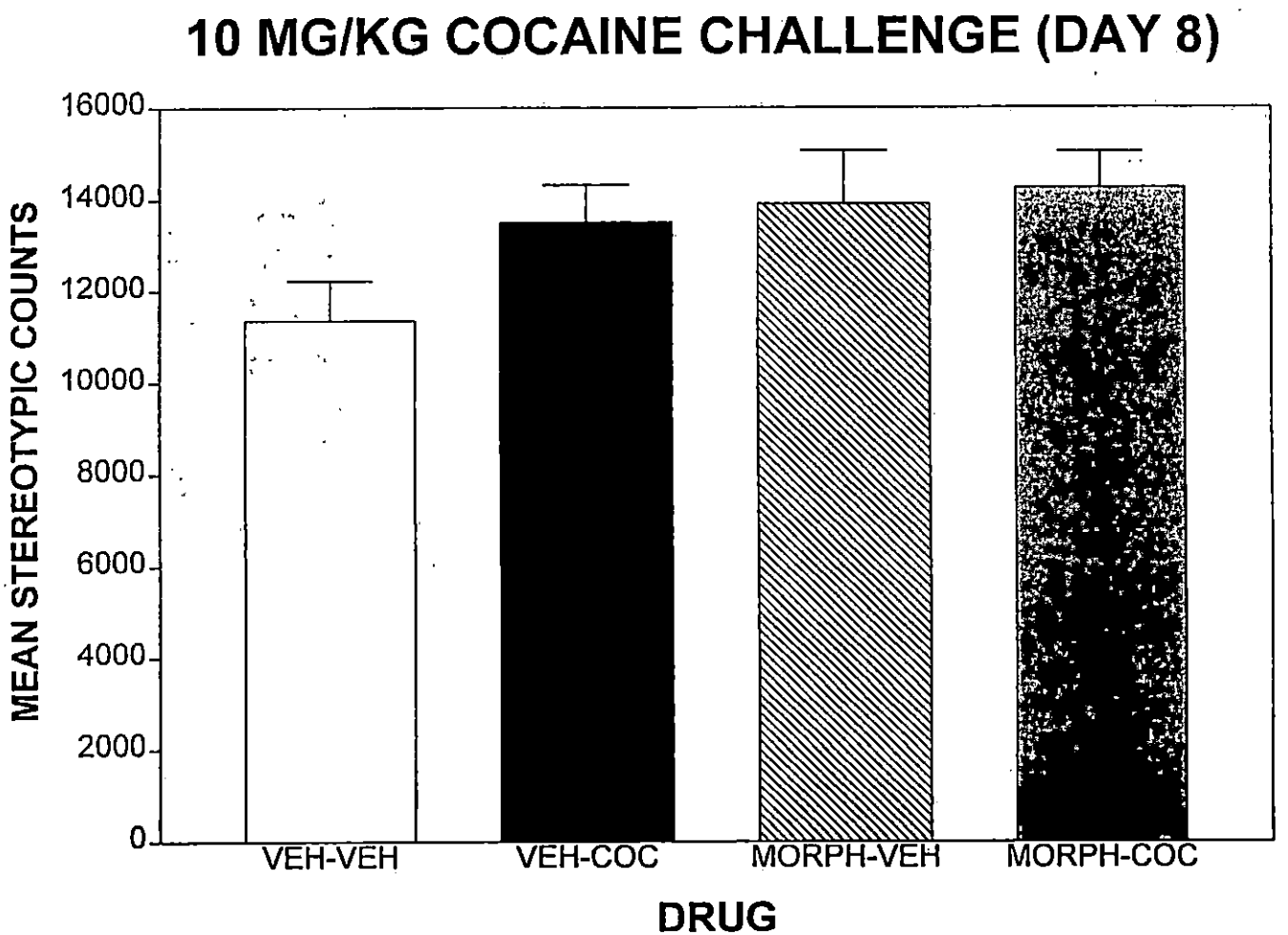

Figure 10. Mean stereotypic counts after a challenge injection of cocaine (10 mg/kg) for each of the four pretreatment groups over the 120 min session.

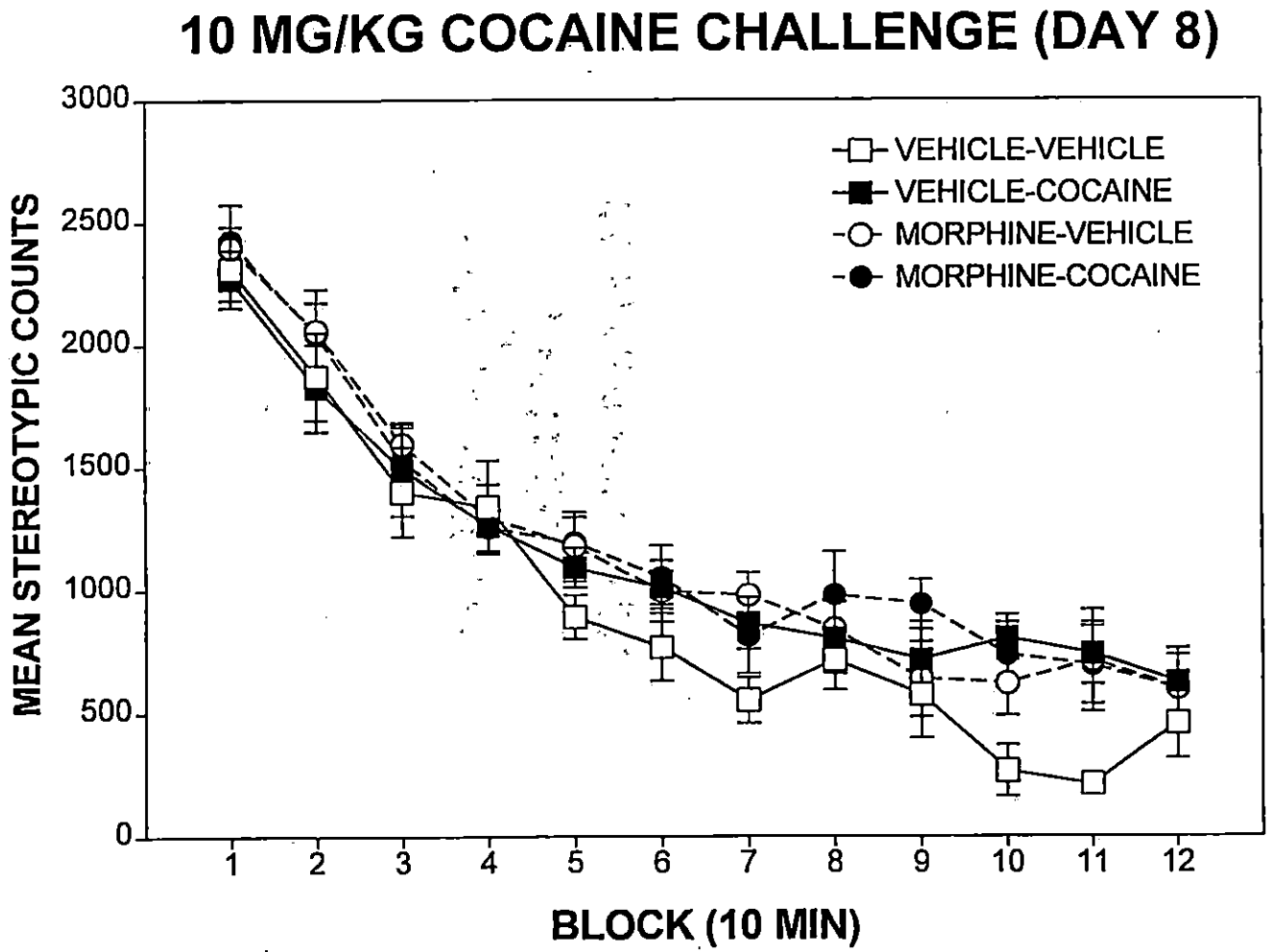

Figure 11. Mean stereotypic counts after a challenge injection of cocaine (10 mg/kg) for each of the four pretreatment groups across the twelve 10 min blocks within the 120 min session.

There were no overall significant differences between the groups. As depicted in Figure 11, there was an overall decrease in activity for all groups over the twelve 10 min blocks [block effect: $F(11, 308) = 97.16$, $p < .0001$] (See Appendix A, Table 6).

Rearing:

The mean number of rears for the four pretreatment groups during the 120 min cocaine challenge are shown in Figure 12 while the within session mean number of rears for the four pretreatment groups is illustrated in Figure 13. Like stereotypy, there were no overall significant differences between the groups. However, animals pretreated with cocaine appeared to be more active than animals with no previous exposure to cocaine on some blocks [block effect $F(11, 308) = 63.04$, $p < .0001$; Cocaine x Block interaction: $F(11, 308) = 2.42$, $p < .01$] (See Appendix A, Table 7).

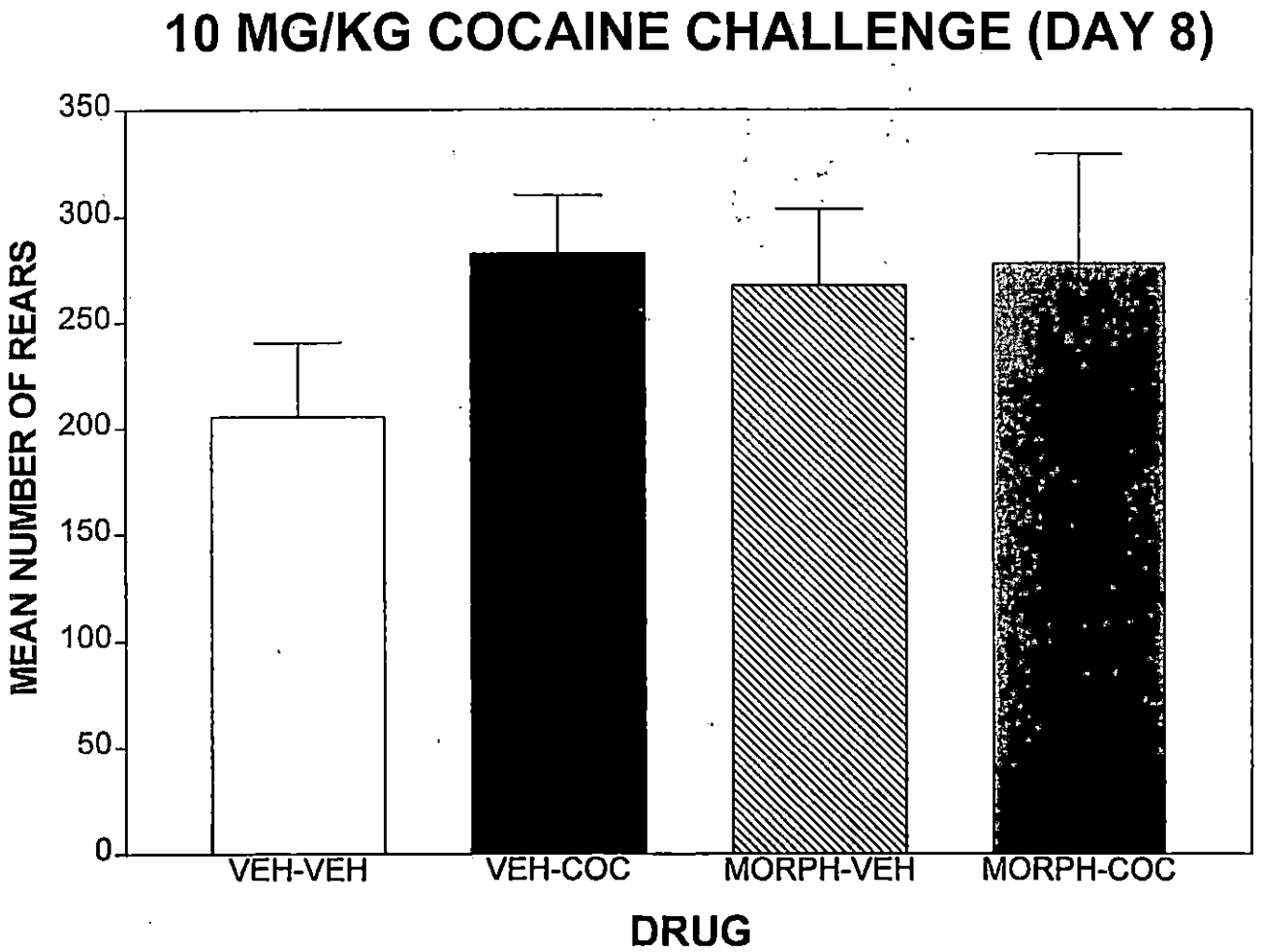

Figure 12. Mean number of rears after a challenge injection of cocaine (10 mg/kg) for each of the four pretreatment groups over the 120 min session.

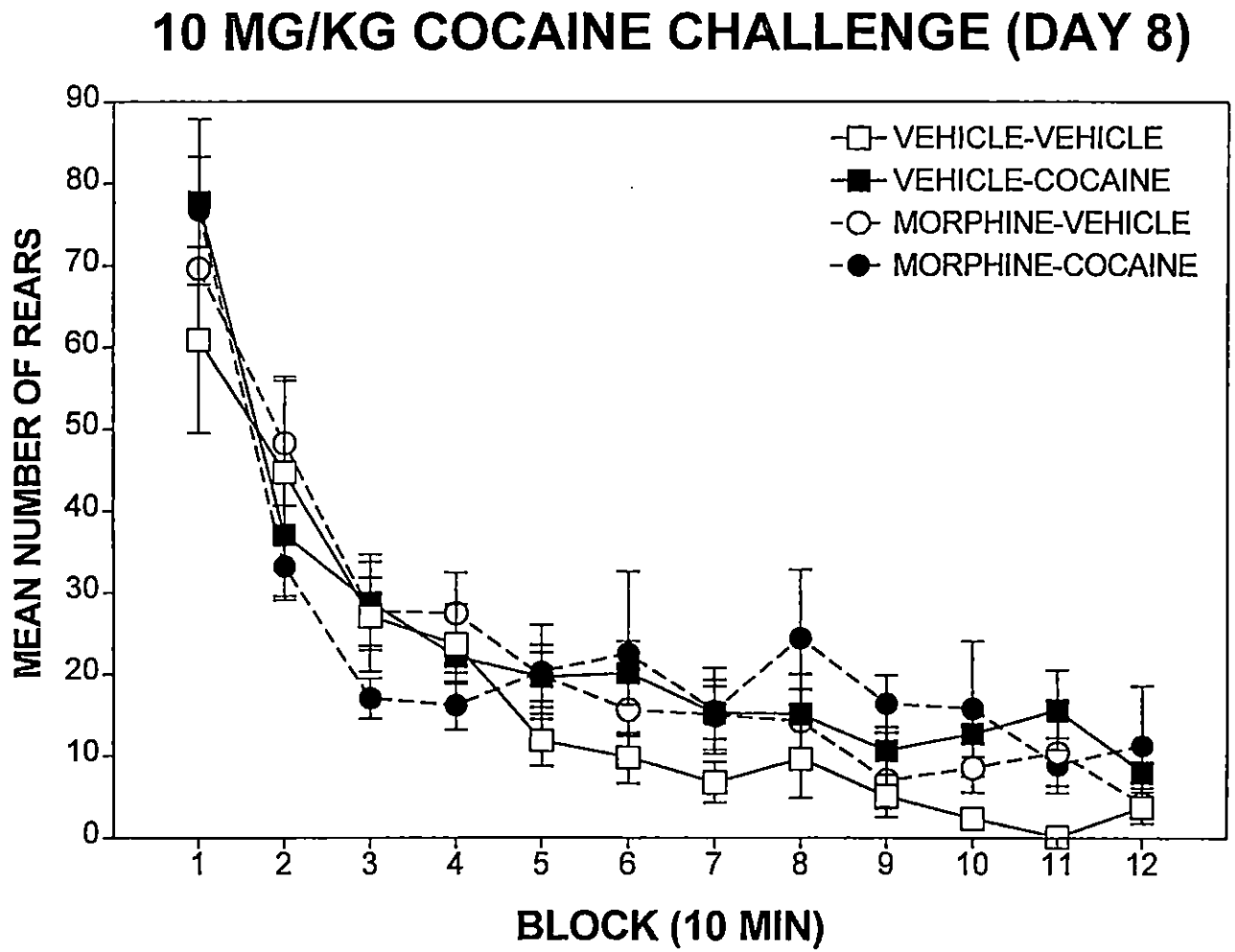

Figure 13. Mean number of rears after a challenge injection of cocaine (10 mg/kg) for each of the four pretreatment groups across the twelve 10 min blocks within the 120 min session.

CHAPTER 4

DISCUSSION

Previous research has examined behavioral sensitization through two distinct methods. First, the observation that injections of a psychostimulant lead to a progressive increase in the dependent measure, typically locomotion, demonstrates behavioral sensitization. An additional technique to measure behavioral sensitization has been to compare animals exposed to a drug versus animals with no previous drug exposure after a "challenge" injection of the drug. To demonstrate sensitization, the pre-exposed drug group should display a significantly augmented behavioral response to the challenge injection when compared to the group that received no prior drug experience. Indeed, several studies have used both methods of demonstrating behavioral sensitization.

The current study investigated the development and expression of behavioral sensitization via the two previously described measures over three dependent measures: distance traveled, rearing, and stereotypy.

I. Behavioral sensitization to cocaine

Consistent with previous research, animals receiving cocaine displayed an increase in mean distance traveled from day 1 to day 7 (e.g., sensitization) (Mattingly et al., 1994; Mattingly et al., 1996; Stewart & Badiani, 1993) but not on the other dependent measures. In addition, on day 8, animals with pre-exposure to cocaine

demonstrated a significantly greater response only on one dependent measure, mean distance traveled, to the cocaine challenge compare to vehicle treated animals (Mattingly et al., 1994; Mattingly et al., 1996).

II. Behavioral sensitization to morphine

Surprisingly, animals that received morphine did not show the same progressive increase in activity over the seven pretreatment days as animals that received cocaine did. However, animals that received morphine pretreatments demonstrated a greater response in mean distance traveled on the cocaine challenge test compared to animals that did not receive morphine during pretreatment, suggesting morphine produced cross-sensitization to cocaine. This finding is consistent with previous literature that demonstrates that dopamine agonists (i.e., cocaine and amphetamine) cross-sensitize to morphine (Vanderschuren et al., 1997; Vandershuren et al., 1999; Vezina et al., 1989). In addition, the increased response to cocaine present in animals that received morphine during pretreatment suggests that cocaine and morphine may have similar physiological effects.

III. Effect of the combination of cocaine and morphine

As mentioned previously, research has demonstrated that the combined administration of cocaine and a mu opioid agonist may increase the behavioral and rewarding effects of these drugs compared to either drug singularly (Kunko et al.,

1998; Kimmel et al., 1997b; Rowlett & Wolverton, 1997; Rowlett et al., 1998; Duvauchelle et al., 1998). Indeed, the main purpose of the current experiment was to determine whether the combination of cocaine and morphine interact and activate locomotor activity in an additive or synergistic way. The results of this study suggest that the combination of cocaine and morphine do not act in an additive way. During the pretreatment phase, the addition of cocaine to morphine did not increase the mean distance traveled compared to animals that received only morphine. Additional evidence is provided by the day 8 cocaine challenge day. Indeed, on day 8, the combination of the two drugs, cocaine and morphine, did not significantly increase activity beyond either drug alone. This finding is consistent with previous research in which doses of cocaine and morphine, individually subthreshold for the induction of behavioral sensitization, did not combine in an additive manner (Mattingly et al., 1999).

IV. Summary and conclusions

As noted previously, cocaine and opioid agonists are commonly abused in a drug combination known as "speedball" (Kosten et al., 1986). At present, little is known of the behavioral and neuropharmacological mechanisms mediating the attractiveness of the drug combination. Evidence from clinical studies suggests that stimulant/opioid combinations may enhance the euphoric effects of each drug and/or attenuate the negative side effects of each drug (Hunt et al., 1984; Strug et al., 1985).

At present, conflicting evidence exists in the preclinical studies with rats and monkeys. Although some studies have reported an enhanced reinforcing effect of stimulant/opioid combinations compared to either drug alone (Rowlett & Woolverton, 1997; Duvauchelle, Sapoznik, & Kornetsky, 1998; Ranaldi & Wise, 2000), others have reported no significant enhancements (Mello et al., 1995). Similarly, the subjective effects of stimulant and opioid compounds do not appear to be additive as measured by drug discrimination tasks (Lamas et al., 1998; Negus et al., 1998a; Negus et al., 1998b). Taken together, these findings suggest that the attractiveness and abuse liability of speedball combinations is not simply due to enhancement of each drugs subjective and reinforcing effects.

The present findings are consistent with this conclusion. That is, although repeated morphine treatments produced cross-sensitization to cocaine, the addition of morphine to cocaine did not enhance the development of behavioral sensitization to cocaine. This finding is consistent with previous literature in this laboratory using combinations of doses of cocaine and morphine subthreshold for the development of behavioral sensitization (Mattingly et al., 1999).

To the extent that the development of behavioral sensitization is a valid model for the induction of craving (see Robinson & Berridge, 1993; Robinson & Berridge, 2000; Robinson & Berridge, 2001), the current findings suggest the combination of cocaine and morphine does not induce any greater craving than either drug alone. However, additional research is needed to further evaluate the long-term effects of

this drug combination. For example, the current study used a short pretreatment phase (7 days) and a short withdrawal interval (24 hrs). Sensitization to either cocaine or morphine, however is known to persist for weeks or months following drug withdrawal. Whether the combination of cocaine and morphine would affect the long-term persistence of behavioral sensitization is unknown.

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APPENDIX A

ANOVA SUMMARY TABLES

AND COUNTERBALANCING

Table 2

Summary of Analysis of Variance Performed on Mean Distance Traveled: Pretreatment Days 1-7

Source	Df	MS	F	nu^2
Between Groups				
Morphine (M)	1	10860594	9.71***	0.26
Cocaine (C)	1	37355314	33.41****	0.54
MxC	1	5300087	4.74*	0.14
Error	28	1118212		
Within Groups				
Day (D)	6	550527	3.01**	0.10
MxD	6	230568	1.26	0.04
CxD	6	662735	3.63**	0.11
MxCxD	6	223666	1.22	0.04
Error	168	182747		
Block (B)	11	24358653	248.36****	0.90
MxB	11	155296	1.58	0.05
CxB	11	4723013	48.15****	0.63
MxCxB	11	96765	0.99	0.03
Error	308	98080		
DxB	66	42358	1.32*	0.05
MxDxB	66	44220	1.38*	0.05
CxDxB	66	87069	2.72****	0.09
MxCxDxB	66	23397	0.73	0.03
Error	1848	32009		

p.0001****
p.001***
p.01**
p.05*

Table 3

Summary of Analysis of Variance Performed on Mean Stereotypic Count: Pretreatment Days 1-7

Source	Df	MS	F	nu^2
Between Groups				
Morphine (M)	1	45379113	19.72****	0.41
Cocaine (C)	1	94534878	41.08****	0.59
MxC	1	20403107	8.87**	0.24
Error	28	2301080		
Within Groups				
Day (D)	6	269548	0.57	0.02
MxD	6	439401	0.93	0.03
CxD	6	812547	1.73	0.06
MxCxD	6	921707	1.96	0.07
Error	168	470170		
Block (B)	11	58467039	364.48****	0.93
MxB	11	353557	2.20**	0.07
CxB	11	5612641	34.99****	0.56
MxCxB	11	632003	3.94****	0.12
Error	308	160414		
DxB	66	223347	2.30****	0.08
MxDxB	66	108511	1.12	0.04
CxDxB	66	110593	1.14	0.04
MxCxDxB	66	106842	1.10	0.04
Error	1848	97041		

p.0001****
p.001***
p.01**
p.05*

Table 4

Summary of Analysis of Variance Performed on Mean Number of Rears: Pretreatment Days 1-7

Source	Df	MS	F	nu^2
Between Groups				
Morphine (M)	1	15405	4.28*	0.13
Cocaine (C)	1	154533	42.97****	0.61
MxC	1	7577	2.11	0.07
Error	28	3597		
Within Groups				
Day (D)	6	4034	5.28****	0.16
MxD	6	484	0.63	0.02
CxD	6	1189	1.56	0.05
MxCxD	6	2499	3.27**	0.10
Error	168	764		
Block (B)	11	89016	180.09****	0.87
MxB	11	219	0.44	0.02
CxB	11	14780	29.90****	0.52
MxCxB	11	699	1.42	0.05
Error	308	494		
DxB	66	701	4.67****	0.14
MxDxB	66	138	0.92	0.03
CxDxB	66	241	1.60****	0.05
MxCxDxB	66	174	1.16	0.04
Error	1848	150		

p.0001****
p.001***
p.01**
p.05*

Table 5

Summary of Analysis of Variance Performed on Mean Distance Traveled: Cocaine Challenge Day 8

Source	Df	MS	F	nu^2
Between Groups				
Morphine (M)	1	788692	4.69*	0.14
Cocaine (C)	1	17666395	10.50**	0.27
MxC	1	161270	0.96	0.03
Error	28	168271		
Within Groups				
Block (B)	11	5743693	105.48****	0.79
MxB	11	28758	0.53	0.02
CxB	11	154499	2.84**	0.09
MxCxB	11	26452	0.49	0.02
Error	308	54452		

p.0001****
p.001***
p.01**
p.05*

Table 6

Summary of Analysis of Variance Performed on Mean Stereotypic Count: Cocaine Challenge Day 8

Source	Df	MS	F	nu^2
Between Groups				
Morphine (M)	1	1807445	3.20	0.10
Cocaine (C)	1	1038232	1.84	0.06
MxC	1	542327	0.96	0.03
Error	28	565079		
Within Groups				
Block (B)	11	10481411	97.16****	0.78
MxB	11	38128	0.35	0.01
CxB	11	114071	1.06	0.04
MxCxB	11	108786	1.01	0.03
Error	308	107872		

p.0001****
p.001***
p.01**
p.05*

Table 7

Summary of Analysis of Variance Performed on Mean Number of Rears: Cocaine Challenge Day 8

Source	Df	MS	F	nu^2
Between Groups				
Morphine (M)	1	546	0.56	0.02
Cocaine (C)	1	1276	1.30	0.04
MxC	1	748	0.76	0.03
Error	28	982		
Within Groups				
Block (B)	11	10607	63.04****	0.69
MxB	11	89	0.53	0.02
CxB	11	408	2.42**	0.08
MxCxB	11	90	0.54	0.02
Error	308	168		

p.0001****
p.001***
p.01**
p.05*

Table 8

COUNTERBALANCING

Squad #	Subject #	Pretreatment Group	Chamber #
1	1	Vehicle-Vehicle	1
1	2	Vehicle-Cocaine	2
1	3	Morphine-Vehicle	3
1	4	Morphine-Cocaine	4
2	5	Morphine-Vehicle	1
2	6	Morphine-Cocaine	2
2	7	Vehicle-Vehicle	3
2	8	Vehicle-Cocaine	4
3	9	Vehicle-Cocaine	1
3	10	Vehicle-Vehicle	2
3	11	Morphine-Cocaine	3
3	12	Morphine-Vehicle	4
4	13	Morphine-Cocaine	1
4	14	Morphine-Vehicle	2
4	15	Vehicle-Cocaine	3
4	16	Vehicle-Vehicle	4
5	17	Vehicle-Vehicle	1
5	18	Vehicle-Cocaine	2
5	19	Morphine-Vehicle	3
5	20	Morphine-Cocaine	4
6	21	Morphine-Vehicle	1
6	22	Morphine-Cocaine	2
6	23	Vehicle-Vehicle	3
6	24	Vehicle-Cocaine	4
7	25	Vehicle-Cocaine	1
7	26	Vehicle-Vehicle	2
7	27	Morphine-Cocaine	3
7	28	Morphine-Vehicle	4
8	29	Morphine-Cocaine	1
8	30	Morphine-Vehicle	2
8	31	Vehicle-Cocaine	3
8	32	Vehicle-Vehicle	4